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FINAL REPORT TO THE OFFICE OF NAVAL RESEARCH.(U)
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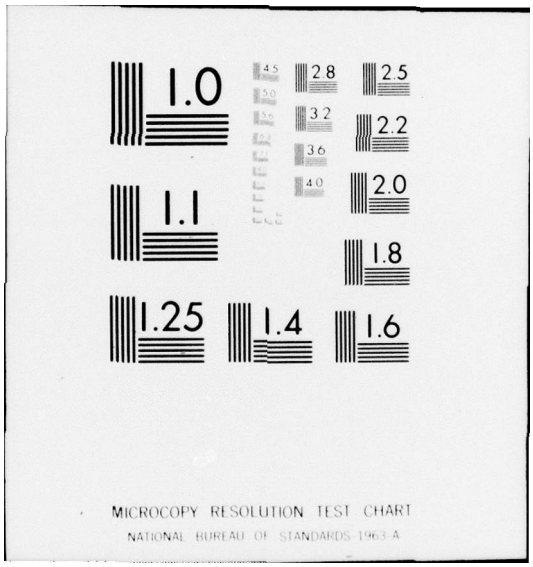
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

1) The spin-label studies of hemoglobin provide many basic examples of how the spin-label technique may be applied to biological systems. It became clear that covalently attached spin labels are extremely sensitive probes when differences between a "strongly-immobilized" spectrum and one with "weaker immobilization" were correlated with the function of a biological model. These techniques are now readily →

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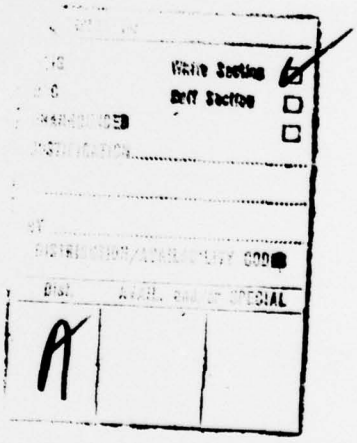
20. applicable to analyses of other isolated proteins.

2) We developed a model, referred to as the concerted transition model, that accounts for several biochemical and biophysical functional properties of hemoglobin, and hemoglobin mutants.

3) We continued development of spin-label magnetic resonance methods for determination of the rates of very slow molecular motion (molecular motions with correlations in the range of 10^{-3} to 10^{-7} sec).



FINAL REPORT TO THE

OFFICE OF NAVAL RESEARCH

This report includes the following grants:

N00014-67-A-0112-0045 - "Spin-Labeled Protein Crystals"

April 1, 1969 to March 31, 1970
renewal, April 1, 1970 to March 31, 1971
renewal, April 1, 1971 to March 31, 1972
renewal, May 1, 1972 to April 30, 1973
renewal, May 1, 1973 to April 30, 1974

N00014-67-A-0012-0045 - "Study of Slow Molecular Motions Using Spin Labels"

renewal, May 1, 1974 to April 30, 1975

N00014-75-C-0869 - "Study of Slow Molecular Motions Using Spin Labels"

renewal, May 1, 1975 to April 30, 1976

SUBMITTED BY: Harden M. McConnell, Principal Investigator
Department of Chemistry, Stanford University
Stanford, California 94305

In the first three years of ONR support (April 1, 1969 through March 31, 1972, under grant no. N0014-67-A-0112-0045) research was carried out in two principal areas: (1) studies of the molecular basis of cooperative oxygen binding to hemoglobin, and (2) determination of spin-label orientations in protein single crystals. Our knowledge of the precise structure of spin-labeled hemoglobin, and its slight deviations from the structure of normal hemoglobin, was greatly advanced by the X-ray crystallographic analysis completed on our crystals by Mr. Keith Moffat at MRC, Cambridge. A detailed knowledge of the position of the spin label in the hemoglobin tetramer increased our understanding of the molecular interactions which give rise to the observed paramagnetic resonance spectral changes on deoxygenation of fully oxygenated tetramer. Our previous spin-label investigations of hemoglobin demonstrated the existence of structures intermediate between the fully oxy- and fully deoxy- structures. This is the basis of "cooperativity", by which one subunit of the tetramer transmits information about its state to another subunit.

In a further test of the ability of spin-labeled hemoglobin to exhibit normal structural changes between the oxygenated and deoxygenated states, we studied the effect of inositol hexasphosphate (IHP) on the oxygen binding curve of hemoglobin. IHP binds only to deoxy-hemoglobin and facilitates the deoxygenation

process. Thus, IHP is able to sense the structural difference between oxy- and deoxy- hemoglobins. Our finding that spin-labeled deoxyhemoglobin interacts normally with IHP supports our previous conclusion that the spin-labeled protein is a valid model of natural hemoglobin.

The spin-label studies of hemoglobin supported by ONR provide many basic examples of how the spin-label technique may be applied to biological systems. It became clear that covalently attached spin labels are extremely sensitive probes when differences between a "strongly-immobilized" spectrum and one with "weaker immobilization" were correlated with the function of a biological molecule. A summary of our spin-label work in several fields and the variety of techniques developed from our hemoglobin work in the first three years of ONR support are described in references 1, 2, and 3. These techniques are now readily applicable to analyses of other isolated proteins.

Publications supported by ONR in these first three years are references 1, 2, 3, 4, 5, and 6.

In the intermediate period of ONR support (May 1, 1972 through April 30, 1975, under grant no. N00014-67-A-0112-0045) we developed a model, referred to as the concerted transition model, that accounts for several biochemical and biophysical functional properties of hemoglobin, and hemoglobin mutants.

This model has the special feature that the α-heme groups are treated as having physical properties distinct from the β-heme groups; moreover the effects of well-known mutations (HbA, HB Chesapeake, Kempsey, Yakima, Kansas) are shown to affect primarily the oxygen affinities of the α or β heme groups, as well as the allosteric equilibrium constant L. As far as we know, our work represents the most systematic attempt to account quantitatively for the functional properties of such a large number of hemoglobin molecules. Further, as far as we know, there is no significant experimental evidence against the validity of this model. We therefore regard our work in this area as highly successful. Publications describing this model are references 7, 8, 9, 10, 11, 12, and 13.

In the final year of ONR support (May 1, 1975 through April 31, 1976, under grant no. N00014-75-C-0869) we continued development of spin-label magnetic resonance methods for determination of the rates of very slow molecular motion (molecular motions with correlations in the range of 10^{-3} to 10^{-7} sec), work which we began during the last year of ONR grant no. N00014-67-A-0112-0045. In some of this work we used "saturation transfer spectroscopy", a recently-developed method which greatly expanded the use of the spin-label technique since many "collective motions" of biological membranes are thought to have correlation

times within the exclusive range saturation transfer spectroscopy measures, 10^{-3} to 10^{-7} sec. (Here "collective motion" is simply a loose term implying that many molecules, or many subunits of a polymer, move together in a concerted way.)

Theoretical work on the study of the rates of very slow molecular motion can be applied to many biophysical problems. The methods developed are applicable to biological membranes or model membranes (phospholipid bilayers) in addition to other important systems such as liquid crystals and polymers.

Publications in the area of spin-label magnetic resonance studies supported by ONR include references 14, 15, and 16.

A copy of the abstract of the most recent article by Thomas, Dalton, and Hyde (16) follows. Reprints will be forwarded to ONR when they become available.

In summary, the use of spin-labels has already lead to a number of significant biophysical discoveries, especially in the area of biological membranes. The range of problems that can be studied using the spin-label technique has been greatly expanded by our studies conducted with ONR support.

Harden M. McConnell
Principal Investigator

PUBLICATIONS SUPPORTED IN PART OF WHOLE BY ONR

1. "Applications of spin-labels in pharmacology," W. L. Hubbell and H. M. McConnell, Proc. Fourth Int'l. Congress on Pharmacology (Schwebe and Co., Basel, Switzerland) (1970).

2. "Physics and chemistry of spin labels," H. M. McConnell and B. G. McFarland, Quart. Rev. Biophys. 3, 91-136 (1970).

3. "Molecular motion in biological membranes," H. M. McConnell, in The Neurosciences: A Study Program, ed. F. O. Schmitt, (Rockefeller University Press, New York) pp. 697-706 (1970).

4. "Magnetic resonance studies of anesthetics in cytomembranes," W. L. Hubbell, J. C. Metcalfe, and H. M. McConnell, Brit. J. of Pharmacology 35, 374 (1969).

5. "The molecular basis of fluidity in membranes," B. G. McFarland, in Proc. of Meeting, Titisee, Germany, October 1971.

6. "Spin label orientation at the active site of α-chymotrypsin in single crystals," L. J. Berliner and H. M. McConnell, Biochem. Biophys. Res. Comm. 43, 651 (1971).

7. "Spin label studies of cooperative oxygen binding to hemoglobin," H. M. McConnell, Ann. Rev. Biochem. 40, 227 (1971).

8. "Spin-labeled hemoglobin in solution, polycrystalline suspensions, and single crystals," R. T. Ogata and H. M. McConnell, in Probes of Structures and Functions of Macromolecules and Membranes. Vol. II - Probes of Enzymes and Hemoproteins, p. 241 (1971).

9. "Mechanism of cooperative oxygen binding to hemoglobin," R. T. Ogata and H. M. McConnell, Proc. Nat. Acad. Sci. 69, 335 (1972).

10. "Binding of triphosphate spin labels to hemoglobin Kempsey," R. T. Ogata, H. M. McConnell and R. T. Jones, Biochem. Biophys. Res. Comm. 47 157 (1972).

11. "States of hemoglobin in solution," R. T. Ogata and H. M. McConnell, Biochemistry 11, 4792 (1972).

12. "The binding of a spin-label triphosphate to hemoglobin Yakima and Kansas," Patrick Coleman, submitted for publication to Biochemistry.

13. "Triphosphate spin label studies of allosteric interactions in hemoglobin," R. T. Ogata and H. M. McConnell, Ann. N. Y. Acad. Sci. 222, 56-64 (1974).

14. "New EPR methods for the study of very slow motion: application to spin-labeled hemoglobin," James S. Hyde and David D. Thomas, Ann. N. Y. Acad. Sci. 222, 680-692 (1973).

15. "Motion of subfragment-1 in myosin and its supramolecular complexes: saturation transfer electron paramagnetic resonance," David D. Thomas, John C. Seidl, James S. Hyde and John Gergely, Proc. Nat. Acad. Sci. 72, 1729-1733 (1975).

16. "Rotational diffusion studies by passage saturation transfer electron paramagnetic resonance," David D. Thomas, Larry R. Dalton, James S. Hyde, and Departments of Radiology and Biochemistry, The Medical College of Wisconsin, submitted for publication to J. of Chem. Physics.

Rotational Diffusion Studied by Passage Saturation Transfer Electron Paramagnetic Resonance

Abstract

A comprehensive description is given of instrumental and theoretical methods employed to make accurate measurements of rotational correlation times using passage saturation transfer electron paramagnetic resonance (ST-EPR). Saturation transfer methods extend by several orders of magnitude the sensitivity of EPR to very slow motion; for example, for nitroxide spin labels, correlation times as long as 10^{-3} sec become accessible to measurement. Two ST-EPR detection schemes are discussed in detail: dispersion, detected 90° out-of-phase with respect to the 100 kHz field modulation, and absorption, detected 90° out-of-phase with respect to the second harmonic of the 50 kHz field modulation. The sensitivities of these configurations are illustrated with experimental spectra obtained from a system obeying isotropic Brownian rotational diffusion; namely, maleimide spin labeled human oxyhemoglobin in aqueous glycerol solutions. Two theoretical approaches, one employing coupled Bloch equations and the other utilizing the stochastic Liouville equation for the density matrix with the orientation variables treated by transition rate matrix or orthogonal eigenfunction expansion methods, are in excellent agreement with each other and with model system spectra. Both experimental and theoretical spectra depend on a number of relaxation processes other than rotational diffusion; consequently, considerable care must be taken to ensure the accuracy of measured rotational correlation times. Although the absorption method is currently the more sensitive and convenient one to apply with most conventional (commercial) spectrometers, the dispersion ST-EPR method is potentially more powerful, providing strong motivation for future technological efforts to decrease noise levels in dispersion experiments.